UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES
Antimicrobials Division

December 21, 2004

MEMORANDUM:

Efficacy Review EPA Reg. No. 1677-164 *Tsunami 100*
DP Barcode DP307171

From: Nancy Whyte, Efficacy Team Leader (Acting)
Efficacy Evaluation Team
Product Science Branch
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December 23, 2004

To: Marshall Swindell/Tony Kish
PM Team 33
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Antimicrobials Division (7510C)

Thru: Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

Applicant: Ecolab Inc.
370 North Wabasha Street
St. Paul, MN 55102

Formulation Label: **% by wt.**

Active Ingredient(s)

Peroxyacetic acid	15.%
Hydrogen peroxide	11.0%
Other ingredients	74.0%
Total	100.0%

I. Background:

The product, Tsunami 100 (EPA Reg. No. 1677-164), is an EPA-approved product for controlling the growth of non-public health microorganisms that cause plant decay and spoilage of post-harvest and processed fruits and vegetables. The product is for use in commercial and institutional environments. The applicant requested an amendment to the registration of this

product to add claims of effectiveness as a water additive for reducing pathogens in fruit and vegetable processing waters. The study was conducted at Ecolab Inc.'s Research and Development Center, located at 840 Sibley Memorial Highway in Mendota Heights, MN 55118.

This data package contained a letter from EPA to the applicant (dated November 27, 2001) that forwarded EPA's review of a testing protocol (dated October 19, 2001), a copy of an Ecolab Inc. protocol titled "Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Processing Waters for Fruit and Vegetables" (dated November 8, 2002), one study (MRID No. 463439-01), a Statement of No Data Confidentiality Claims for the study, and the proposed label.

The initial review of this amendment request was conducted by DynCorp Systems and & Solutions LLC, Alexandria, Va.

II. Use Directions:

The product is designed to be used as a water additive for reducing the levels of specific pathogens in fruit and vegetable processing waters. Directions on the proposed label provided the following information regarding preparation and use of the product for this application:

For Batch Systems: Ensure that water is being mixed in the processing vessel. Add 18-47.8 mL of the product per 100 liters of water, or 1 fluid ounce of the product per 43.5-16.3 gallons of water. Measure the residual peroxyacetic acid concentration in the water using a Test Kit. Adjust dose as needed. Allow a 1.5 minute mixing time. The target range of residual peroxyacetic acid in the use solution is 30-80 ppm.

For Continuous Systems, Initial Dose: Ensure that water is being mixed in the processing vessel and piping. Add 18-47.8 mL of the product per 100 mL of water, or 1 fluid ounce of the product per 43.5-16.3 gallons of water. Measure the residual peroxyacetic acid concentration in the water using a Test Kit. Adjust dose as needed. Allow a 1.5 minute mixing time. The target range of residual peroxyacetic acid in the use solution is 30-80 ppm.

For Continuous Systems, Continuous Dosing: Meter in the product to achieve 30-80 ppm residual peroxyacetic acid in proportion to the fresh water makeup water added to the system. For example, makeup water flow rates of 43.5-16.3 gallons per minute would require a maximum of 1 fluid ounce (29.6 mL) per minute of the product. Makeup water flow rates of 100 liters per minute would require a maximum of 18-47.8 mL per minute of the product.

The product is also designed to be used for treating fruit and vegetable surfaces and process waters to prevent against plant decay and spoilage. The proposed label directions provided the following information regarding preparation and use of the product for this application: Add 47.8 mL of the product per 100 liters of water, or 1 fluid ounce of the product per 16.4 gallons of water. Spray or submerge fruits and vegetables in the use solution for a minimum of 30 seconds. Allow use solution to drain.

IV. Agency Standards for Proposed Changes:

Water Additive for Reducing Foodborne Pathogenic Bacteria in Fruit and Vegetable Processing Waters

The Agency does not have set guidelines and/or a performance standards for products of this type. In 2001, an expert panel reviewed a draft protocol prepared by Ecolab Inc. In November 2002, Ecolab Inc. revised this protocol in response to comments received from the expert panel. According to this revised protocol, triplicate measurements are to be made of the product. Three strains of *Listeria monocytogenes* (ATCC 49594, 19114, 19116), three strains

of *Escherichia coli* serotype O157:H7 (ATCC 43895, 35150, 43890), and three strains of *Salmonella choleraesuis* subsp. *choleraesuis* (serotype *javiana* ATCC 10721, serotype *newport* ATCC 6962, serotype *typhimurium*, ATCC 13311) must be tested. The average inoculum population of each challenge microorganism must be at least 10^6 CFU/mL. Populations of each challenge microorganism must be reduced by >99.9% (relative to the inoculum populations). These standards are presented in "Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Processing Waters for Fruit and Vegetables" (developed by Ecolab, Inc.; dated November 8, 2002).

IV. Comments on the Submitted Efficacy Study:

MRID 463439-01 "Tsunami 100 – To Reduce Foodborne Pathogenic Bacteria in Processing Waters for Fruit and Vegetables (EPA Reg. No. 1677-164)," by Petra Hochmuth. Study conducted at Ecolab Inc.'s Research and Development Center. Study completion date – August 12, 2004. Study Number 0400010.

This study was conducted against three strains of *Listeria monocytogenes* (ATCC 49594, 19114, 19116), three strains of *Escherichia coli* serotype O157:H7 (ATCC 43895, 35150, 43890), and three strains of *Salmonella choleraesuis* subsp. *choleraesuis* (serotype *javiana* ATCC 10721, serotype *newport* ATCC 6962, serotype *typhimurium* ATCC 13311). Three lots (Lot Nos. 121903A, 021304B, 021304C) of the product, Tsunami 100, were tested using the Ecolab Inc. protocol titled "Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Processing Waters for Fruit and Vegetables". All three product lots were at least 60 days old at the time of testing. Use solutions of the product were prepared at 30±10 ppm total peracid (as peroxyacetic acid) in 400 ppm synthetic hard water (titrated at 400 ppm) with 1% sterile mixed vegetable juice added to the dilution water. Each test system was transferred daily in culture media at least 4 times but less than 15 times, with no more than 1 day missed per 7-day period. The growth from the culture medium agar slants was used to prepare French slants. French slants, prepared using phosphate buffered dilution water (PBDW), were each inoculated with 2 mL of the test system. Excess liquid was decanted. French slants were incubated horizontal for 24±4 hours at 35±2°C. Growth from the French slants was harvested. Test system suspensions were filtered, and equal volumes of each strain of *Listeria monocytogenes* were combined in a sterile container. The same was done for *Escherichia coli* O157:H7 and *Salmonella choleraesuis*. The density of each suspension was adjusted using PBDW. For each test system, 99-mL aliquots of the use solution were transferred to sterile, 250-mL Erlenmeyer flasks and placed in a water bath at 25±2°C. One-mL bacterial suspension was added to each flask. After a 90-second exposure period, 1 mL of the bacterium-product mixture was transferred to 9 mL of 0.1% sodium thiosulfate to neutralize. The neutralizer tubes were mixed and, where appropriate, serially diluted in PBDW. Selected aliquots were plated in Tryptic Soy Agar plates for the *Escherichia coli* O157:H7 and *Salmonella choleraesuis* test systems. Brain Heart Infusion Agar plates were used for the *Listeria monocytogenes* test system. All plates were incubated for 48±4 hours at 35±2°C and colonies were counted. Controls included those for inoculum population determination, purity, sterility, and neutralization. The concentration of residual peroxyacetic acid was measured and determined to be 26-36 ppm.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Results:

MRID Number	Organism	Lot No.	Time	Average No. Surviving	Microbes Initially Present	Percent Reduction
				(CFU/mL)		
463439-01	*Listeria monocytogenes*	121903A 021304B 021304C	90 sec. 90 sec. 90 sec.	7.6×10^4 7.0×10^4 7.6×10^4	8.8×10^7 8.8×10^7 8.8×10^7	99.914 99.920 99.914
463439-01	*Escherichia coli* O157:H7	121903A 021304B 021304C	90 sec. 90 sec. 90 sec.	1.0×10^1 1.0×10^1 1.0×10^1	7.8×10^7 7.8×10^7 7.8×10^7	99.999 99.999 99.999
463439-01	*Salmonella choleraesuis*	121903A 021304B 021304C	90 sec. 90 sec. 90 sec.	1.0×10^1 1.0×10^1 1.0×10^1	9.1×10^7 9.1×10^7 9.1×10^7	99.999 99.999 99.999

V. Comments:

A. Conclusions Regarding Completeness of Protocol (in Response to Changes Requested by the Agency)

In its October 19, 2001 review of Ecolab Inc.'s proposed protocol, the Agency requested that a number of changes be made to the draft protocol. In a memorandum dated November 8, 2002 Ecolab Inc. provided EPA with a revised protocol. The Agency's requested changes to the draft protocol are presented below, along with comments from this reviewer.

1. Inactivating Agent - The agent used to inactivate the bactericidal properties of the antimicrobial agent should be specified rather than leaving it to the analyst to choose.

Reviewer's Comment: The revised protocol (dated November 8, 2002) recommends using 0.1% sodium thiosulfate as the neutralizer.

2. Tempered Agar - When plating the antimicrobial agent working solution, the temperature of the Trypticase Soy Agar or the Brain Heart Infusion [Agar] should be specified in the protocol.

Reviewer's Comment: The revised protocol (dated November 8, 2002) specifies a 46±2°C temperature for the agar plates during the plating process.

3. "Recycled Water" - The proposed use of "recycled water" was mentioned in the protocol. However, the registrant is required to generate data from a challenged water including a soil load. The proposed test method does not mention any soil loading to reflect that which will almost certainly be present in such water.

Reviewer's Comment: The revised protocol (dated November 8, 2002) specifies testing in the presence of 1% sterile mixed vegetable juice.

4. Dilution Water - Water used in the dilutions of the antimicrobial agents should be reflective of what is used in the field, not sterilized deionized water as stated in the proposed protocol. It is highly unlikely that such water is used in the field for processing raw fruits and vegetables. Any water under field conditions will have a certain number of dissolved chemicals in it and this should be reflected in the proposed test protocol by incorporating the use of water with a specified and clearly stated level of hardness. Many standard test methods require the use of water with 200-400 ppm hardness of $CaCO_3$.

Reviewer's Comment: The revised protocol (dated November 8, 2002) specifies testing in the presence of 200-400 ppm hard water.

5. Reducing Populations - The end-user should not imply that reducing populations of pathogens in the water will also significantly reduce populations on the product. This rather definitive statement should be changed. When referring to populations, replace the term "numbers" with a more precise concentration or population. This avoids confusion with strain numbers.

Reviewer's Comment: The revised protocol (dated November 8, 2002) clearly refers to testing of processing waters (as opposed to testing of fruit or vegetable surfaces).

B. Conclusions Regarding Test Results

1. The submitted efficacy data (MRID No. 463439-01) support the use of a 30 ppm residual peroxyacetic acid use solution of the product, Tsunami 100, for reducing *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* ssp. within 90 seconds in fruit and vegetable processing waters. A reduction of 99.9% in bacterial counts was observed. Three product lots were tested. Inoculum populations were at least 10^6 CFU/mL. Neutralization testing indicated that the average plate counts for Controls A and B were within a 0.5 $\log_{10}$ of the average plate count for Control C. Sterility controls showed no growth. Purity controls were reported to be typical of the test systems.

V. Labeling:

1. The proposed label claims that a 30-80 ppm residual peroxyacetic acid use solution of the product, Tsunami 100, will provide a 99.9% reduction against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* ssp. when a 1.5 minute mixing time is used. Data provided by the applicant support this claim. All dosages on the proposed label appear to be correct.

2. Certain language on the proposed label does not clearly describe what level/type of efficacy the product provides. The Agency has approved this product for controlling the growth of non-public health microorganisms that cause decay and spoilage in process waters and on the surfaces of fruits and vegetables [see the last-accepted label dated October 29, 2001]. With the submission of data reviewed as part of this data package, the applicant has now demonstrated efficacy of the product in reducing 99.9% of certain pathogens in fruit and vegetable processing waters (i.e., *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella choleraesuis*). It is important to note that the

applicant has not provided data to show that the product reduces the number of pathogens on fruit and vegetable surfaces. The applicant must make the following changes to the proposed label:

- Revise the language under the product name to read: "Water Additive for Pathogen Reduction in Fruit and Vegetable Processing Waters. Also a Water Additive for Controlling the Growth of Spoilage and Decay Causing, Non-public Health Microorganisms on Fruit and Vegetable Surfaces and Processing Waters."

- Revise the language following the "Environmental Hazards" section to read: "Used as directed, Tsunami 100 reduces >99.9% of the pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. in fruit and vegetable processing waters. Used as directed, Tsunami 100 provides control of spoilage and decay causing, non-public health organisms present in processing waters and on the surface of post-harvest, fresh-cut and processed fruit and vegetables.

- Add the following sentence to the "For Treatment of Fruit and Vegetable Surfaces and Process Waters" section: "This product is not intended for control of any public health organisms on fruit and vegetable surfaces."

3. The proposed label now claims that the product, Tsunami 100, is effective in treating fruit and vegetable surfaces at a 30-second contact time [see page 2 of the proposed label]. The applicant has not provided data to support this new claim. The last accepted label specified a 45-second contact time. The applicant must provide appropriate data to support the 30-second contact time, or revise the label to indicate a 45-second contact time.

4. The applicant must make the following changes to the proposed label, as appropriate:

- Under the "First Aid" section, change "Take of contaminated clothing." to read "Take off contaminated clothing."

- Under the "First Aid" section, change "Do not anything by mouth to an unconscious person." to read "Do not give anything by mouth to an unconscious person."